REMARKS

I. Amendments to the Claims

Claims 5, 6, and 10 have been cancelled.

Claims 1 and 4 have been amended. Support for the amendments is found in the Specification at page 17, lines 29-31 and page 24 beginning at line 17.

Claim 1 has also been amended to recite a correlation step. Support for this amendment is found in the Specification and Example 3.

New claim 12 has been added. Support for claim 12 is found in claim 1.

New claim 13 has been added. Support for claim 13 is found in claim 4.

No new matter has been added.

II. Indefiniteness

The Examiner rejects claims 1 and 4 for reciting that R represent a 25-hydroxylated side group of vitamin D_2 or vitamin D_3 because the Examiner alleges that these vitamins do not have hydroxyl groups at position 25. Applicants submit that the claims recite that R is the side chain portion of a 25-hydroxylated vitamin D_2 or 25-hydroxylated vitamin D_3 moiety and that this is not indefinite. See also, structure (II) at page 7, which illustrates a "25-hydroxylated side group of vitamin D_3" at the position of R. Applicants request that the rejection be withdrawn.

The Examiner rejects claim 1 on the grounds that it is allegedly unclear that 1α, 25-dihydroxy vitamin D is detectable in a sample that also contains 25-hydroxy vitamin D. The Specification discloses that the test practiced with labeled 25-hydroxy vitamin D could also be applied to 1α,25-dihydroxy vitamin D, if there is a "binding protein or a receptor or antibody . . . which specifically recognizes the 1α,25-dihydroxy vitamin D analog." (Specification, page 17, lines

29-31). Applicants have also amended the claims to recite detecting "25-dihydroxy vitamin D or 1α, 25-dihydroxy vitamin D or both" or "25-dihydroxy vitamin D and a 1α, 25-dihydroxy vitamin D metabolite." (Claims 1 and 4, respectively). Furthermore, there are methods of separating 25-hydroxy vitamin D from 1α, 25-dihydroxy vitamin D prior to running the samples in the presently claimed method, as discussed in the specification at page 24 beginning at line 17. One of skill would recognize that either possibility could be practiced with the claimed method based on their knowledge of ELISA-type assays. Applicants submit that the amendment overcomes the Examiner's rejection. Applicants request that the rejection be withdrawn.

The Examiner rejects claims 1-3 and 10 as omitting the essential step of correlating the detection of vitamin D displacement with the presence/amount of 25-hydroxy or 1α, 25-dihydroxy vitamin D metabolite in the sample. Claim 1 has been amended to recite a correlation step, thereby overcoming the rejection. Applicants request that the rejection be withdrawn.

III. Enablement

The Examiner rejects claims 1-3 and 11 as not enabled. The Examiner takes the position that it is not possible to measure the amount of 1α, 25-hydroxy vitamin D metabolite that is present in a sample by a competitive binding assay when 25-hydroxy vitamin D metabolite is also present in the sample. In particular, the Examiner contends that, because the amount of 1α, 25-dihydroxy vitamin D metabolite is negligibly low as compared to 25-hydroxy vitamin D metabolite, the displacement of vitamin D metabolite from vitamin D binding protein would be mainly due to 25-hydroxy vitamin D metabolite and cannot be correlated to the amount of 1α, 25-dihydroxy vitamin D metabolite in the sample. Applicants submit that the Examiner has misunderstood the implication of the invention.

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17, lines 29-31). Applicants note that there are antibodies specific for a 1α,25-dihydroxy vitamin D analog (See attached papers, page 15). Specifically, Applicants point out that the assays sold by ALPCO are Applicants' own products, as ALPCO is the United States distributor for Applicants. Furthermore, the protocol of the ALPCO assay (pages 10-12) is identical to Example 9 of the present Specification. Also, as discussed above, there are known methods of separating 1α, 25-hydroxy vitamin D and a 25-hydroxy vitamin D metabolite. Applicants submit that this disclosure in the Specification, as evidenced by the market acceptance of a product within the claims, allows one of skill to make and use the claimed invention. Applicants request that the rejection be withdrawn.

IV. Obviousness

The Examiner rejects claims 1-8 and 11 as obvious over Holick et al.. The Examiner also rejects claim 9 as obvious over Holick et al. in view of DeLuca et al.. Applicants respectfully submit that any *prima facie* showing of obviousness is rebutted by the fact that the Holick reference is not enabling for an assay of a 25-hydroxy vitamin D metabolite as discussed below.

In the parent patent application (U.S. Patent Text from U.S. 6,787,660) Applicants compared the disclosure of Holick against a method of measuring the amount of a 25-hydroxy vitamin D metabolite or a 1α,25-hydroxy vitamin D metabolite disclosed in the present invention. Based on Holick, one of skill would expect a displacement ratio of 1:11, whereas Applicants show that the ratio is 1:1. Based on this difference and the disclosure in Holick, Applicants have asserted in the past that Holick did not make 25-hydroxy vitamin D displacement ligands (i.e. compound C or D) and one of skill would not be able to make and use the 25-hydroxy vitamin D displacement ligands described by Holick. In the Amendment dated February 15, 2003, beginning on page 4, Applicants wrote:

> The instant invention relates to a method for detecting a vitamin D analogue that contains a hydroxyl group at the 25 position. Holick '127 does not disclose all of the elements of the instantly claimed invention.

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First, for the Examiner's benefit, the attachment that the Examiner was indicated was missing from the response of August 7, 2002 showing the ring structures of the compounds of Holick '127 and the instant invention is attached. As was pointed out in that response, the "biotin conjugate" shown on page 17 in Holick '127 is not biotin.

Second, . . . intervening chain [between the 'biotin' and the vitamin D metabolite in Figure 6] is a long ester to a 25-OH vitamin D moiety. The chemical formula shows an ester and the chemical reaction shown in Figure 6 produces an ester. As explained on page 7, lines 4 to 14 of the instant invention, human blood serum and plasma contain esterases, which attack ester chains such as is shown in Figure 6 in Holick '127.

Third, Applicants assert that the NMR and UV data present in Holick '127 merely shows that there was a vitamin D ring system present in its disclosure but there is no proof that the 25 OH-group was present in any one of the target compounds. In other words, the disclosure in Holick '127 is not enabled. The previous publications cited by the Examiner (Ray et al., Steroids, Vol. 60(8), pp.530-533 (1995) and Swamy et al., Protein Expression and Purification, Vol. 6(2), pp. 185-188, (1995)) allegedly describe the aminopropylation of 25 hydroxy vitamin D. However, the relevant starting material 25-hydroxyvitamin-D3-3α-3-aminopropylether (compound 4a) was never obtained and used in any one of the cited publications.

The newly recited references both refer to a method of synthesis described by Ray et al. in Biochemistry Vol. 30, pp. 4809-4813 (1991), which has been cited in the instant application. In the Ray et al. reference, cyanoethylated 25-OH vitamin D is reduced to the corresponding amine by means of LiAlH4-AlCl3 (Note that Ray et al. is cited in Cite 8 in Swamy et al. and Cite 5 in Roy et al.). However, the reduction of cyanoethylated 25-OH vitamin D by LiALH4-AlCl3 not only reduces the cyano group but also reduces the 25-hydroxyl group of the vitamin D if it is not protected as claimed in the instant invention. Thus, it appears that the reduction of the 25-hydroxyl group by LiAlH4 was apparently not noted by Ray et al. This is proven by the fact that Holick '127 requires an eleven-fold surplus of biotinylated target compound to replace one molecule of 25-OH vitamin D on the vitamin D binding protein.

In other words, Holick '127 used, in its displacement studies, biotinylated vitamin D instead of biotinylated 25 OH-vitamin D. Thus, all of the binding and ELISA studies in Holick '127 were made with derivatives of vitamin-D rather than 25 OH-vitamin D. There is no proof in Roy et al. or Swamy et al. that the 25-OH group was present in any one of the synthesized vitamin D derivatives. No mass spectrum was reported to prove that the target compound had indeed the

> calculated molecular weight, no IR data showing the O-H stretch is reported, no elemental analysis was reported showing the composition was reported, and the H-NMR data are all not conclusive as was pointed out in the response of August 7, 2002. Thus, it is maintained that neither Holick '127 nor any of Ray's previous publications contain an enabling disclosure. Holick '127 did not make biotinylated 25 OH-vitamin D as it asserts. The structural analyses of Holick '127 are useless in the regard that there is no showing that they made biotinylated 25 OH-vitamin D. The biological tests, in contrast, show that Holick '127 did not make biotinylated 25 OH-vitamin D because Holick '127 requires an eleven-fold increase in binding tests between vitamin-D (having no 25-hydroxyl group) and human plasma vitamin-D-binding protein.
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> The Scatchard plots submitted in the last response as well as the chemical formulas submitted in the last response are again submitted herewith. In the Scatchard plots [see figure 15 of the present application], the displacement efficiencies of 25-hydroxy vitamin D (as claimed in claims 25 or 26), a vitamin D dimer (as claimed in claims 25 or 26), and 3H-25-OH-Vitamin-D on vitamin D binding protein from goat serum are shown. Please see example 3, (iv), line 18 et seq. on page 24 in the written description for the experimental protocol followed. As can be seen in the Scatchard plots, the displacement efficiencies of the compounds of the instant invention were all close to 1, whereas compound C in Holick '127 could only displace the corresponding tritiated compound from human vitamin D binding protein when it was present at an eleven-fold excess. In other words, compound C in Holick '127 is not the 25-OH-vitamin D compound. The disclosure of Holick '127 is not enabled for making this compound. The rejection is inapposite. Withdrawal of the rejection is warranted and respectfully requested.

Applicants submit that these comments clearly explain why Holick does not enable assay for a 25-hydroxy vitamin D compound. Applicants submit that "prior art is not enabling so as to be anticipating if it does not enable a person of ordinary skill in the art to carry out the invention. " *Impax Laboratories Inc. v. Aventis Pharmaceuticals Inc.,* 81 USPQ2d 1001, 1013 (Fed. Cir. 2006). Thus, Applicants submit that the present invention is not obvious in view of Holick.

Applicants also point out that the present invention demonstrates unexpected benefits as compared to Holick. Applicants submit that the difference in displacement ratios of the displacement ligand of the invention compared to compound C of Holick establishes that they are NOT the same and therefore the present invention is not anticipated by/or obvious over Holick's disclosure.

However, if the Examiner wishes to take the alternative view that Holick discloses the same compounds, the present invention has an unexpected and unexplained 1:1 displacement ratio rather than a 1:11 displacement ratio, allowing the present invention to be more precise than that of Holick. (See Specification, page 4, line 31-32). Additionally, the present invention is able to determine the amount of a 25-hydroxy vitamin D metabolite at a lower concentration than that disclosed in Holick. (See Specification, figure 5C discussed at page 35, disclosing sensitivity below 10 pg of vitamin D metabolite in the sample and compare to Holick, page 22, line 10, where the lowest amount of "25-0H-D_3" in the standard curve is 20 pg). Moreover, Applicants use one third of the amount of biotinylated compound per well in comparison to what Holick discloses is "minimum amount[] of . . . compounds (C) [biotinylated 25-0H vitamin D] required to obtain yellow color," i.e., the minimum amount of compound required to read the assay. (See, Specification page 23, line 15 compared to Holick, page 20, line 3). Therefore, the present invention unexpectedly saves reagent and has a lower limit of detection. Thus, Applicants submit that the unexpected benefits of the present invention overcome any *prima facie* showing of obviousness.

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

CONCLUSION

In view of the above remarks, it is believed that claims are allowable.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Dr. Mark Nuell Reg. No. 36,623 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

Dated: October 31, 2008

Respectfully submitted,

By _____

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Attachments: ALPCO ELISA Product Material